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Anti-Endothelial Cell Antibodies are not frequently elevated in hospitalized patients with COVID-19

Brandon Michael Henry[1,2], *Stefanie W. Benoit*[2,3], *Jens Vikse*[4], *Emmanuel Favaloro*[5], *Justin L. Benoit*[6], *Giuseppe Lippi*[7]

[1]Disease Intervention & Prevention and Population Health Programs, Texas Biomedical Research Institute, San Antonio, TX, USA; [2]Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, OH, USA; [3]Department of Pediatrics, University of Cincinnati, College of Medicine, OH, USA; [4]Clinical Immunology Unit, Stavanger University Hospital, Stavanger, Norway; [5]Haematology, Sydney Centres for Thrombosis and Haemostasis, Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology, Westmead Hospital, Westmead NSW, Australia; [6]Department of Emergency Medicine, University of Cincinnati, Cincinnati, OH, USA; [7]Section of Clinical Biochemistry, Department of Neuroscience, Biomedicine and Movement, University of Verona, Verona, Italy

To the Editor,

Coronavirus disease 2019 (COVID-19), the pandemic disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been clearly linked to a hypercoagulable state especially in severe illness, which predisposes to development of pulmonary and systemic arterial and venous thromboembolism, being ultimately associated with poor outcomes (1). Now called COVID-19-associated coagulopathy (CAC), the mechanisms underlying this pro-thrombotic condition have yet to be fully elucidated (2).

Several studies have reported variable presence of antiphospholipid antibodies (anticardiolipin (aCL) and anti–β2-glycoprotein I (aβ2GPI)) and lupus anticoagulant (3–6), though the clinical significance of these findings remains unknown, as it is not uncommon to see such antibodies transiently during the course of various infections (2). However, the identification of these antibodies in some patients with COVID-19 persuaded us to evaluate the potential for the role of other autoantibodies in COVID-19.

Anti-Endothelial Cell Antibodies (AECA) are a heterogenous group of autoantibodies targeting various endothelial cell antigens or antigens adhering to endothelial cells (7), They are commonly observed in a variety of auto-immune and rheumatologic conditions (7), including Kawasaki disease (AECA present in up to 72%) (8), which offers a presentation similar to COVID-19 associated Multisystem Inflammatory Syndrome in Children (MIS-C). Moreover, the presence of AECA was previously reported in patients recovering from severe acute respiratory syndrome (SARS) in 2005 (9). While the presence of AECA may represent an epiphenomenon of endothelial damage, there is a possibility that pathogenic AECA could be driving the vasculopathy in COVID-19. While the exact pathogenic effects of AECA is not completely understood, it may involve endothelial cell cytotoxicity through complement-dependent or antibody-dependent cell mediated cytotoxicity (10). Furthermore, AECA may induce pro-inflammatory and pro-coagulant effects through endothelial cell activation (with subsequent leukocyte adhesion and cytokine production), tissue factor release, and cleavage and release of heparin sulphate from endothelial cell surface (10). Given the above, we decided to assess AECA status in patients with COVID-19 and their potential contributing role to endothelial injury and CAC.

Adults presenting to the Emergency Department of University of Cincinnati Medical Center with symptoms suggestive of COVID-19 and requiring blood draw for routine management were

preliminarily enrolled in this study. Blood samples were collected under an institutional review board (IRB) waiver of informed consent. Following collection, samples were centrifuged at 2,000 g for 15 min at 4°C within 3 hours of collection, and frozen at -80°C until analysis. Only patients with a positive transcription polymerase chain reaction (RT-PCR) on nasopharyngeal swab obtained for clinical purposes and who were hospitalized following ED presentation were included. Patients with a history of rheumatological or autoimmune diseases were excluded. Patients were classified by disease severity at ED disposition and peak during hospitalization. Severe disease was defined as any level of respiratory support at or beyond non-invasive ventilation or high flow oxygen devices or illness requiring intensive care unit (ICU) admission. Human AECA were measured using an enzyme-linked immunosorbent assay, and interpreted according to manufacturer's guidelines (CUSABIO Technology, Houston, Texas, USA).

A total of 34 consecutive laboratory-confirmed COVID-19 patients were enrolled. One patient had a history of rheumatoid arthritis and was hence excluded, so that the final sample consisted of 33 patients. The characteristics of the cohort are presented in Table 1. Six patients (18.2%) had severe COVID-19 on admission, and another 10 (30.3%) progressed from mild to severe disease during hospitalization. The median time from symptom onset to presentation was 7 days (IQR: 2–10 days) and 12 (36.3%) patients were positive for anti-SARS-CoV-2 IgA at ED presentation.

AECA antibodies were identified in 2/33 (6.0%) of patients. Both patients had moderate disease at presentation and neither progressed to severe disease during hospitalization. One patient was a 66-year-old African-American male and the other was 64-year-old African-American female. Neither patient had positive anti-SARS-CoV-2 serology at ED disposition. One patient developed acute kidney injury, requiring dialysis. Neither patient suffered from arterial or venous thromboembolism during hospitalization.

In conclusion, AECA identification was a relatively infrequent finding in COVID-19 patients on admission, and their presence, albeit in only 2/33 patients, was not associated with disease severity. However, as the autoantibodies were only measured

Table 1. Characteristics of Hospitalized COVID-19 Patients.

Male: n (%)	54.5%
Age (years): median (IQR)	64 (46-70)
Body Mass Index: median (IQR)	28 (24-34)
Race/Ethnicity: n (%)	
Asian	1 (3.0%)
Black	17 (51.5%)
Hispanic	9 (27.3%)
White	6 (18.2%)
Comorbidities: n (%)	
Coronary Artery Disease	7 (21.2%)
Heart Failure	9 (27.3%)
Hypertension	21 (63.6%)
Hyperlipidemia	10 (30.3%)
Diabetes	17 (51.5%)
Chronic Obstructive Pulmonary Disease	8 (24.2%)
Chronic Kidney Disease	6 (18.2%)
Chronic Liver Disease	7 (21.2%)
Cerebrovascular Disease	6 (18.2%)
Previous Thromboembolism	7 (21.2%)
Cancer	4 (21.1%)
Obesity	16 (48.5%)
Current Smoker	9 (27.3%)
Former Smoker	6 (18.2%)
Labs at ED Presentation: Median (IQR)	
White Blood Cell Count ($x10^3/mm^3$)	7.3 (5.5-9.8)
Neutrophil Count ($x10^3/mm^3$)	5.3 (4.1-8.0)
Lymphocyte Count ($x10^3/mm^3$)	1.0 (0.7-1.4)
Platelet Count ($x10^3/mm^3$)	212 (165.5-298.8)
Ferritin (ug/L)	408.0 (117.5-1395.0)
Fibrinogen (g/L)	6.6 (4.8-8.2)
D-dimer (ug/mL FEU)	1.2 (0.7-2.0)
Anti-SARS-CoV-2 IgA+: n (%)	12 (36.3%)
Days from symptom onset to ED presentation: median (IQR)	7 (2-10)
Severe illness at ED Disposition: n (%)	6 (18.2%)
Severe illness at any point during Hospitalization: n (%)	16 (48.5%)

*ED- emergency department, IQR – interquartile range

at admission, we cannot exclude the possibility of pathogenic AECA developing later in the course of disease. AECA can recognize constitutively expressed or cytokine-induced autoantigens (10). In theory, as a severe course of COVID-19 unfolds, the ensuing hyperinflammatory state and the virus-induced direct cytopathic effects may induce damage- and cytokine-mediated expression of endothelial (neo-) antigens, thus leading to AECA production, which in turn propagates vasculopathy and coagulopathy. Further studies using additional methods are needed to evaluate the presence and potential pathogenic role of AECA in later stages of COVID-19, including in patients with MIS-C.

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Correspondence:
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Brandon Michael Henry, MD
Cincinnati Children's Hospital Medical Center
3333 Burnet Ave., Cincinnati, OH, USA 45229
Tel/Fax: 716.598.8610
E-mail: brandon.henry@cchmc.org